Pfeifer's Lab Journal Club

Tufts University
Chemical and Biological Engineering
Presented by: Saba Parsa
PI: Professor Blaine Pfeifer
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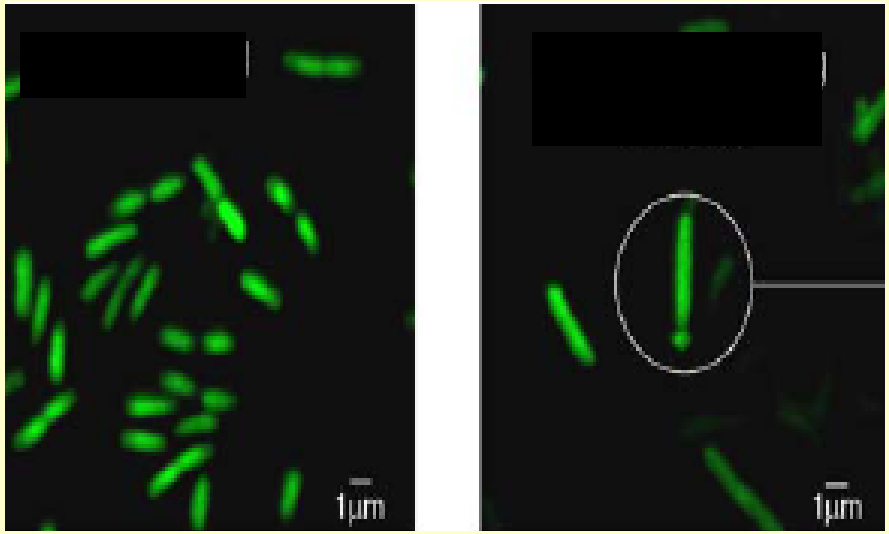
Immune responses elicited by bacterial minicells capable of simultaneous DNA and protein antigen delivery



Giacalone et al. 2006

Outline

- Introduction
- Experiment
- Results
- Summary
- Evaluation
- References

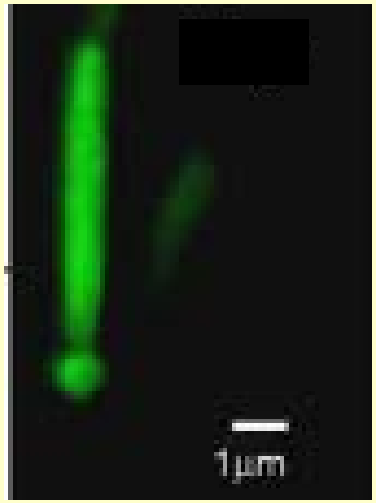
Why minicells & what are they?

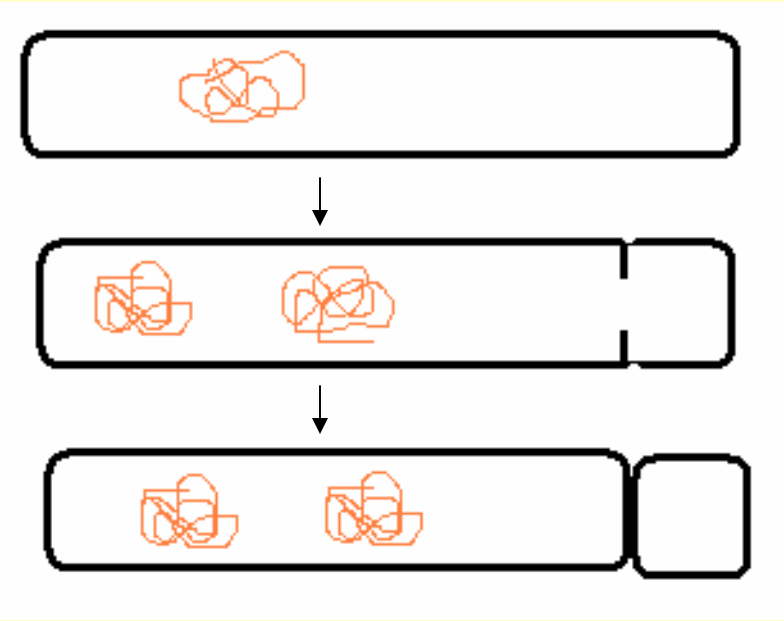
Why Minicells?

- Safety concerns w/ live attenuated pathogens.
- Non-living delivery mechs. better w/ protein & DNA antigen simultaneous delivery.
- Bacterial Minicells: of E. coli strain for effective antigenic immune response.

Bacterial Minicells

- Size: 100-400 nm
- Non-dividing
- Achromosomal
- Produced by most bacterial species
- Metabolically active
- Structural protein and lipid like parental cell





E. coli

Polar cell division

Multinucleated filament & minicell

Giacalone *et. Al, Vaccine, 2006*

Experiment

Experimental Parameters

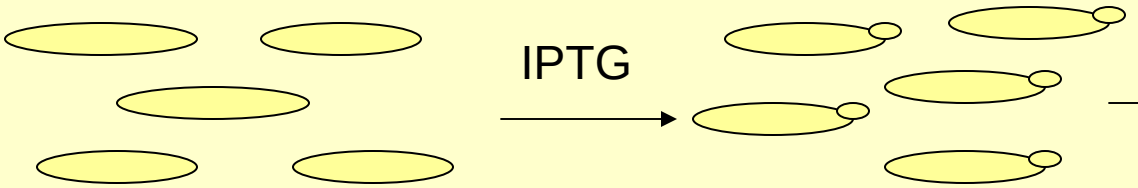
- GFP as general antigen
- Monitor antibody production in sera and mucosa
- Best minicells tested: ***both*** **protein** and **DNA** expressing antigen
- Inducing high levels of IgG (Ab)
- Delivery routes: intramuscular, intranasal, oral

Experimental Design

12 K *E. Coli* strain: MPX1B9

Strains	Characteristics
pRHA-67	Empty
pRHA-67::GFP	GFP Protein
pMJG3	Plasmid DNA
pGFPVAX	Protein & DNA
pGFPM5	GFP His Tag

Minicell Production



IPTG

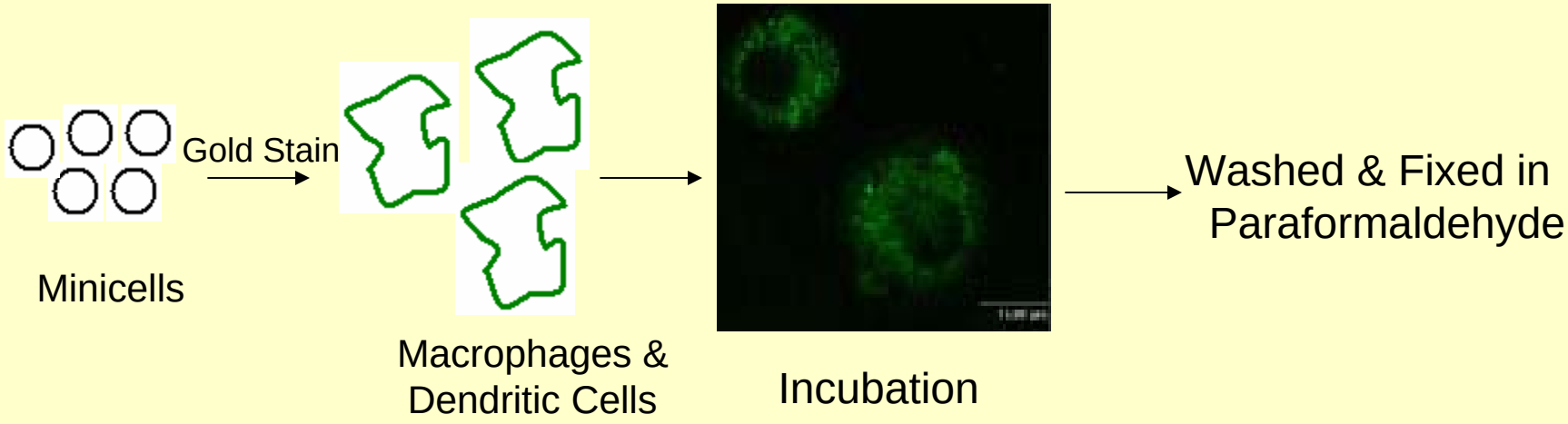
Budding

Harvest & Purify (ciproflaxin)

Western Blot

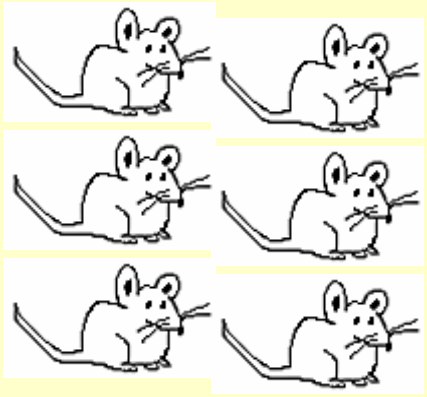
(GFP amount)

Minicell Uptake



Giacalone *et. Al, Vaccine, 2006*

Immunization



Immunized w/
MC & 5 different
plasmids

sacrificed
w/ CO_2

Day 35 or
Day 21

Isolate *sera*: Blood cloth
Isolate *IgA*: lung lavage fluid
small intestine, 15 cm

ELISA

GFP specific Ab screening

Immunization Routes

- Intramuscular: 10^10 MC, 50 ul
- Intranasal: 10^10 MC, 20 ul
- Oral: 10^10 MC, 200ul

Dosage

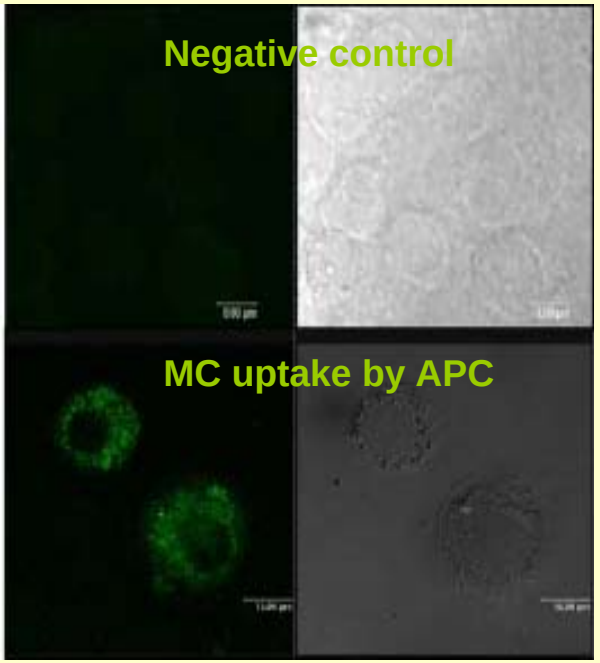
- Triple: t = 0, 14, 28 days
- Single: t = 0 day

Results

Minicell Uptake by APC

Phagocytic cells: minicell uptake

Non-Phagocytic cells: no minicell uptake

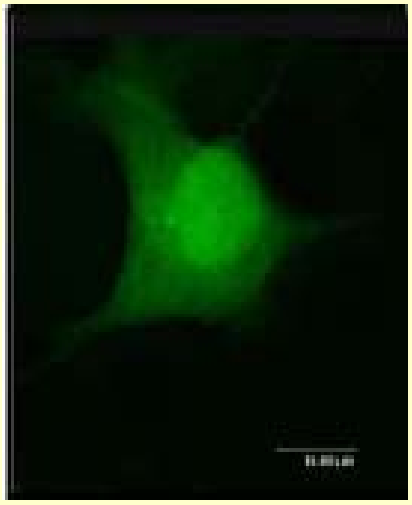


➔ **Ability to deliver hetrologous protein directly to APC (then presented to immune cells).**

Giacalone *et. Al, Vaccine, 2006*

DNA Delivery

- MCs w/ eukaryotic GFP reporter: PMJG3
- Transfected cells: 0.02 – 0.2 %

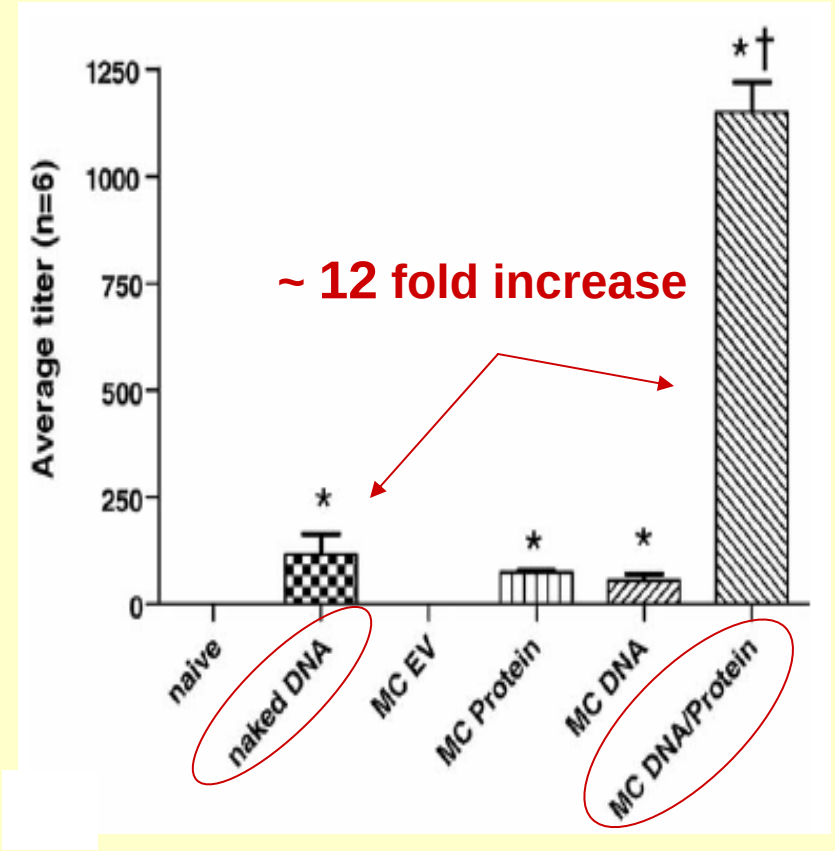


BMDDC cell
expressing GFP transgene
delivered by MCs

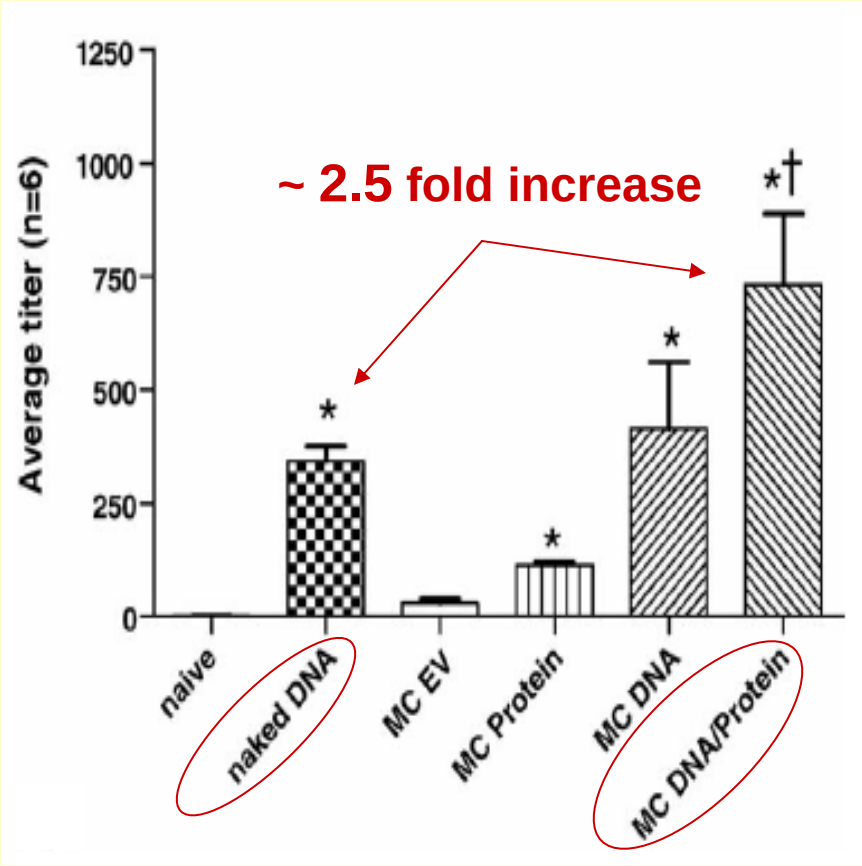
➔ Efficiency not high
but shows ability to **deliver DNA**

Giacalone *et. Al, Vaccine, 2006*

In vivo Study



Triple dose

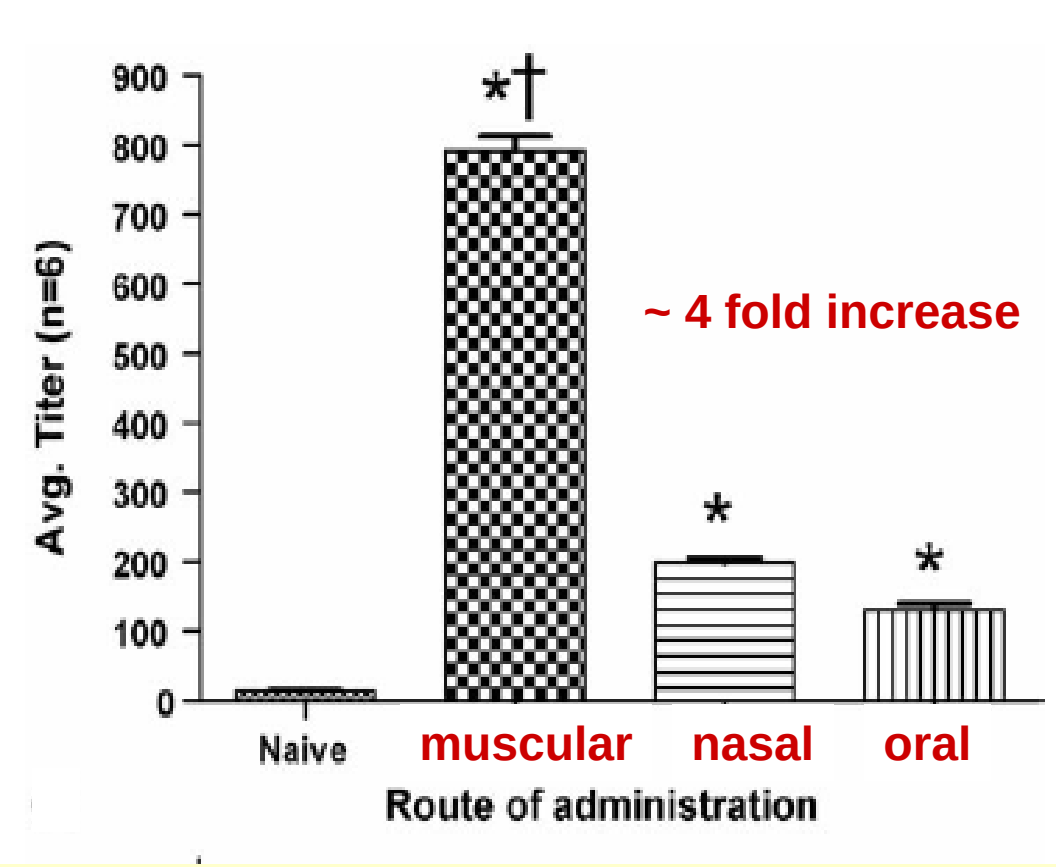


Single dose

Higher dosage ➔ improvement in Ab expression

Giacalone *et. Al, Vaccine, 2006*

Immunization Routes



➔ **Intramuscular delivery** has better Ab expression than nasal and oral.

Giacalone *et. Al, Vaccine, 2006*

Summary
&
Evaluation

Summary

- Ability to deliver hetrologous ***protein*** directly to APC.
- Efficiency not high but shows ability to deliver ***DNA.***
- ***Higher dosage*** ➔ improvement in Ab expression.
- ***Intramuscular delivery*** has better Ab expression than nasal and oral.

Other advantages of minicells

- Better tolerated
- Used for different prime-boost strategies
- E. coli MC is a gut bacteria; survive the harsh environment

Evaluation

- Minicells are safer
- A Novel method
- MCs are convenient
- Minicells formation still vague
- Harvesting methods: differential centrifugation, linear sucrose gradients???
- Efficiency
- More *in vivo* assays
- Why MCs w/ both DNA & protein is better?
- Dosage or timing?
- A more immugenic, cytosolic Ag (instead of GFP)

References

- Giacalone MJ, Sabbadini RA, Chambers AL, Pillai S, McGuire KL: *Immune responses elicited by bacterial minicells capable of simultaneous DNA and protein antigen delivery.* VACCINE 24 (33-34): 6009-6017 AUG 14 2006.
- Sharp MD, Pogliano K: *MinCD-dependent regulation of the polarity of SpoIIIE assembly and DNA transfer*. EMBO J (2002) **21,** 6267–6274.
- Giacalone MJ, Gentile AM, Lovitt BT, Xu T, Surber MW, Sabbadini RA: *The use of bacterial minicells to transfer plasmid DNA to eukaryotic cells.* Cell Microbio. 8(10), 1624-1633: 2006.